PATENT
Docket No. 825-166P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Henrik GAROFF and Peter LILJESTROM

APPLN. NO.: NEW GROUP: Unassigned

FILED: August 10, 1999 EXAMINER: Unassigned

FOR: DNA EXPRESSION SYSTEMS BASED ON ALPHAVIRUS

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, DC 20231

July 10, 2001

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

In the Specification

A printed Sequence Listing is attached. Please append the Sequence Listing to the Specification as a separately page-numbered paper following the claims.

In the Abstract of the Disclosure:

Please insert the Abstract of the disclosure at the end of the application, as follows:

--Abstract of the Disclosure

The disclosure describes recombinant alphavirus RNA molecules and expression of heterologous proteins therefrom in animal cells. Recombinant alphaviruses of the present invention, when made to express an antigenic protein, can be administered as vaccines.--

In the Claims:

Please delete claims 1-41 without prejudice to or disclaimer of the subject matter contained therein.

Please add the following new claims.

--42. A helper cell for producing an infectious, defective alphavirus particle, comprising, in an alphavirus-permissive cell:

(a) an alphavirus replicon RNA, wherein the alphavirus is selected from the group consisting of Sindbis virus and Semliki Forest virus; wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, and a sequence encoding at least one of the alphavirus structural proteins, wherein the replicon RNA furthermore lacks a sequence encoding at least one of the alphavirus structural proteins; and

(b) at least one separate helper RNA encoding the structural protein(s) absent from the replicon RNA, said helper RNA(s) lacking the alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNA(s) produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of helper RNA due to the absence of the structural protein coding sequence in the packaged replicon.

43. The helper cell according to claim 42, wherein said replicon RNA encodes the alphavirus capsid protein, and wherein said at least one separate helper RNA(s) encodes the alphavirus E**1** glycoprotein and the alphavirus E**2** glycoprotein.

44. The helper cell according to claim 42, wherein said alphavirus is Venezuelan Equine Encephalitis virus.

45. The helper cell according to claim 42, wherein at least one of said helper RNA and said replicon RNA includes at least one mutation in E1, E2 or E3.

46. The helper cell according to claim 42, wherein said alphavirus is Semliki Forest virus.

47. The helper cell according to claim 42, wherein said helper RNA and said replicon RNA both include a promoter.

48. The helper cell according to claim 42, wherein said replicon RNA includes a promoter.

49. The helper cell according to claim 42, wherein said inserted heterologous RNA is selected from the group consisting of RNA encoding proteins and RNA encoding peptides.

50. A method of making infectious, defective, alphavirus particles, comprising:

providing a helper cell according to claim 42;

producing said alphavirus particles in said helper cell; and

collecting said alphavirus particles from said cell.

51. The method according to claim 50, wherein said alphavirus replicon RNA and said at least one separate helper RNA are introduced into said helper cell by electroporation.

52. Infectious alphavirus particles produced by the method of claim 50.

53. A pharmaceutical formulation comprising infectious alphavirus particles according to claim 52 in an effective immunogenic amount in a pharmaceutically acceptable carrier.

54. A helper cell for producing an infectious, defective alphavirus particle, comprising an alphavirus-permissive cell transfected with RNAs comprising:

(a) an alphavirus replicon RNA, wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, wherein the replicon RNA furthermore lacks sequences encoding alphavirus structural proteins; and

(b) at least a first and second helper RNAs separate from said replicon RNA and separate from each other, said first and second helper RNAs encoding the structural proteins absent from the replicon RNA;

with said first helper RNA encoding at least one alphavirus structural protein and not encoding at least one other alphavirus structural protein;

with said second helper RNA not encoding said at least one alphavirus structural protein encoded by said

first helper RNA and encoding said at least one other alphavirus structural protein not encoded by said first helper RNA;

and with said first and second helper RNAs lacking the alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNAs produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of the helper RNAs due to the absence of the structural protein coding sequence in the packaged replicon.

55. The helper cell according to claim 54, wherein said first helper RNA encodes both the alphavirus **E1** glycoprotein and the alphavirus **E2** glycoprotein, and wherein said second helper RNA encodes the alphavirus capsid protein.

56. The helper cell according to claim 54, wherein said first helper RNA and said second helper RNA both include a promoter.

57. The helper cell according to claim 54, wherein said replicon RNA includes a promoter.

58. The helper cell according to claim 54, wherein said inserted heterologous RNA is selected from the group consisting of RNA encoding proteins and RNA encoding peptides.

59. A method of making infectious, defective, alphavirus particles, comprising:

providing a helper cell according to claim 54;

producing said alphavirus particles in said helper cell; and then

collecting said alphavirus particles from said cell.

60. The method according to claim 59, wherein said alphavirus replicon RNA and said at least first and second helper RNAs are introduced into said helper cell by electroporation.

61. A cell expressing:

i) a first recombinant RNA molecule comprising an alphavirus RNA genome and an exogenous RNA sequence, wherein said alphavirus RNA genome contains a signal for packaging said recombinant RNA molecule in an alphavirus particle, at least one deletion or stop codon mutation such that at least one structural protein of the alphavirus cannot be translated from said recombinant RNA in said cell, and further wherein said exogenous RNA sequence is operatively inserted into a region of

the alphavirus RNA genome which is non-essential to replication of the recombinant RNA molecule; and

ii) a second recombinant RNA molecule encoding a said at least one structural protein of the alphavirus, wherein said second recombinant RNA molecule lacks a signal for packaging of said second recombinant RNA molecule in an alphavirus particle.

62. A method for producing a recombinant or chimeric alphavirus particle comprising:

i) providing a cell that produces a recombinant or chimeric alphavirus particle, said cell comprising:

a) a first recombinant RNA molecule comprising an alphavirus RNA genome and an exogenous RNA sequence, wherein said alphavirus RNA genome contains a signal for packaging said recombinant RNA molecule in an alphavirus particle, at least one deletion or stop codon mutation such that at least one structural protein of the alphavirus cannot be translated from said recombinant RNA in said cell, and further wherein said exogenous RNA sequence is operatively inserted into a region of the alphavirus RNA genome which is non-essential to replication of the recombinant RNA molecule; and

b) a second recombinant RNA molecule encoding a said at least one structural protein of the alphavirus, wherein said second

recombinant RNA molecule lacks a signal for packaging of said second recombinant RNA molecule in an alphavirus particle;

ii) culturing said cell to produce said recombinant or chimeric alphavirus particles; and

iii) recovering said recombinant or chimeric alphavirus particles from said culture.

63. A helper cell for producing an infectious, defective alphavirus particle, comprising, in an alphavirus-permissive cell:

(a) an alphavirus replicon RNA, wherein the alphavirus is selected from the group consisting of Sindbis virus and Semliki Forest virus; wherein the replicon RNA comprises the alphavirus packaging signal, a heterologous RNA sequence, and a sequence encoding at least one of the alphavirus structural proteins, wherein the replicon RNA furthermore lacks a sequence encoding at least one of the alphavirus structural proteins; and

(b) a helper RNA system comprising helper RNAs encoding the structural protein(s) whose transcripts are absent from or otherwise not functional in the replicon RNA, said helper RNA(s) lacking any alphavirus packaging signal;

wherein the combined expression of the replicon RNA and the helper RNA(s) produces an assembled alphavirus particle which comprises a heterologous RNA sequence, is able to infect a cell, and is unable to complete viral replication in the absence of helper RNA due to the absence of at least one structural protein coding sequence in the packaged replicon.--

REMARKS

Pending Claims

The present application simply continues the prosecution of Application No. 09/371,510. Accordingly, claims 1-41 have been cancelled and replaced by the present claims 42-63. Claims 42-62 correspond to claims 42, 43, 45, 48-50, 53-59, 64, 65 and 68-70 previously pending in the parent application. Claim 63 is newly added for examination.

Amendment of Specification to Follow

The specification of the parent application contains numerous editorial amendments. Applicants will provide a supplemental preliminary amendment providing these amendments in conformity with the new rules of practice.

Sequence Listing

Applicants provide attached hereto a copy of the printed Sequence Listing, first filed on September 17, 1996, in Application No. 07/920,281 that began the present lineage. Pursuant to 37 C.F.R. § 1.821(e), the USPTO is respectfully requested to make use of the CRF filed on September 17, 1996, corresponding to this paper copy of the Sequence Listing. Applicants submit that the CRF filed on September 17, 1996 in the founding Application No. 07/920,281 is

identical to the printed copy of the sequence listing attached hereto. Applicants will make the amendments to the specification to conform the specification to the requirements under 37 C.F.R. § 1.821-1.825 in a Supplemental Preliminary Amendment to follow.

Double Patenting Rejection

Claims 42, 45, 48-50, 53-56, 64-65 and 71-72 of the parent application stand rejected under the judicially-created doctrine of obviousness-type double patenting over claims 13, 18, 24, 33, 37, 39 and 40-43 of U.S. Patent 5,739,026. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested. In particular, Applicants request that the Examiner re-draw the rejection to limit the basis of the rejection to claims 24, 33, 37, 39 and 40-43 of the '026 patent.

Applicants note that the subject matter of the present application is helper cells, methods of using them and products from the cells. Claims 24, 33, 37, 39 and 40-43 of the '026 patent are all directed to cells harboring variously-described helper vectors. On the other hand, claims 13 and 18 of the '026 patent are directed to cells harboring a vector for expressing a desired gene (a "replicon" vector in the terms of the present application). Applicants submit that, whether or not the presently-claimed invention is obvious in view of claims 24, 33, 37, 39 and 40-43 to

cells harboring helper vectors of the '026 application, it is not obvious in view of cells harboring a replicon vector as it is described in claims 13 and 18 of the '026 patent. Applicants also note the Examiner's reasoning that:

> ...the helper cells claimed in the '026 patent are designed to provide the necessary packaging functions ... missing from the alphavirus vector and the cells are used to produce the infectious alphavirus vectors when said vectors are introduced into said cells. (Emphasis added.)

The Examiner reasons that the helper cell claims of the '026 patent are the basis for the rejection. Claims 13 and 18 of the '026 patent are not directed to such helper cells. Accordingly, the present rejection for obviousness-type double patenting should be re-drawn to be based only upon claims 24, 33, 37, 39 and 40-43 of the '026 patent.

Favorable action on the merits of the application is respectfully requested.

If there are any minor matters precluding allowance of the application which may be resolved by a telephone discussion, the Examiner is respectfully requested to contact Mark J. Nuell, Ph.D. (Reg. No. 36,623) at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees

required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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By _____
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DRN/las
825-166P

Attachments:
Mark-up Version Showing Changes
Printed Sequence Listing

MARK-UP VERSION SHOWING CHANGES

In the Abstract of the Disclosure:

The following Abstract of the Disclosure was added to the application:

--Abstract of the Disclosure

The disclosure describes recombinant alphavirus RNA molecules and expression of heterologous proteins therefrom in animal cells. Recombinant alphaviruses of the present invention, when made to express an antigenic protein, can be administered as vaccines.--

In the Claims:

Claims 1-41 were cancelled. New claims 42-63 were added.